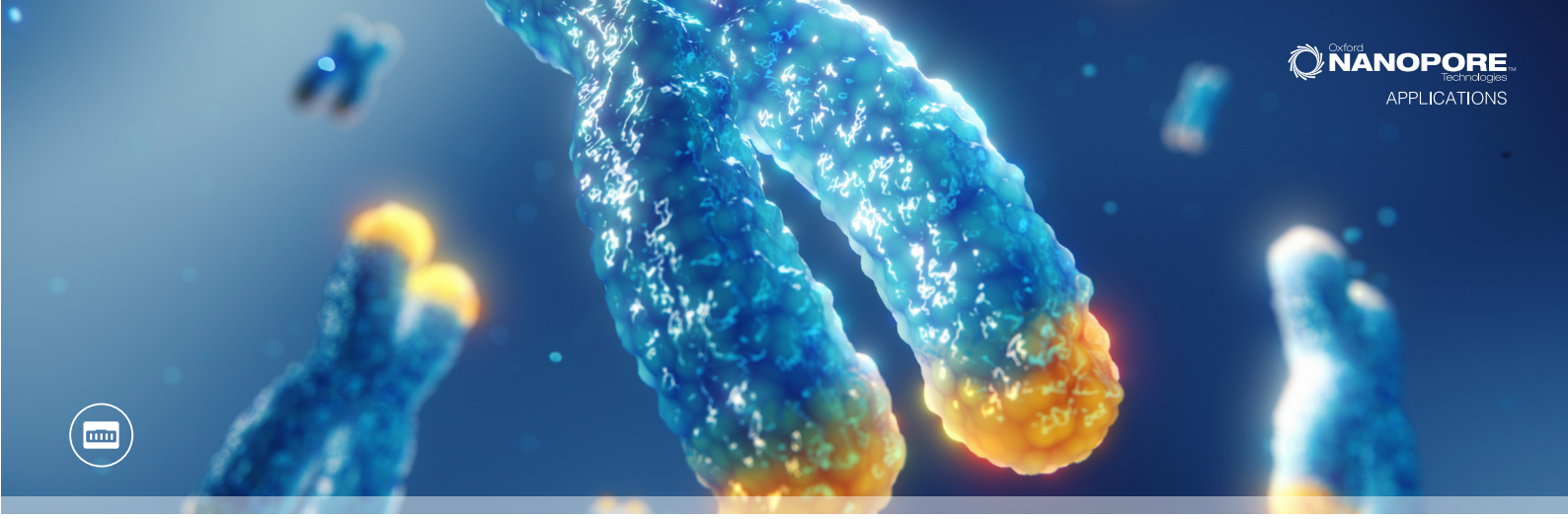

Native telomere sequencing of individual chromosomes using 3' end adapter ligation with Telo-seq

Direct adapter ligation to chromosome ends enables precise telomere mapping, length characterization, and phasing to determine telomere maintenance mechanisms in cancer and telomere shortening with human age

More information at: **www.nanoporetech.com** and **nanoporetech.com/resource-centre** Register your interest at: **https://register.nanoporetech.com/telo-seq**

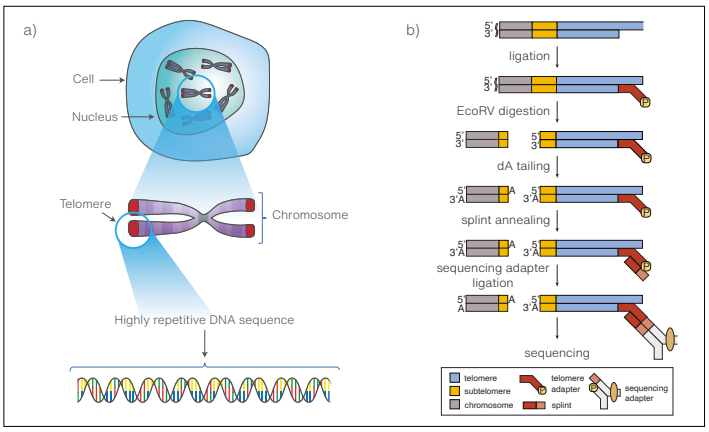

Fig. 1 Overview of Telo-seq workflow, see doi: https://doi.org/10.1101/2023.11.28.569082

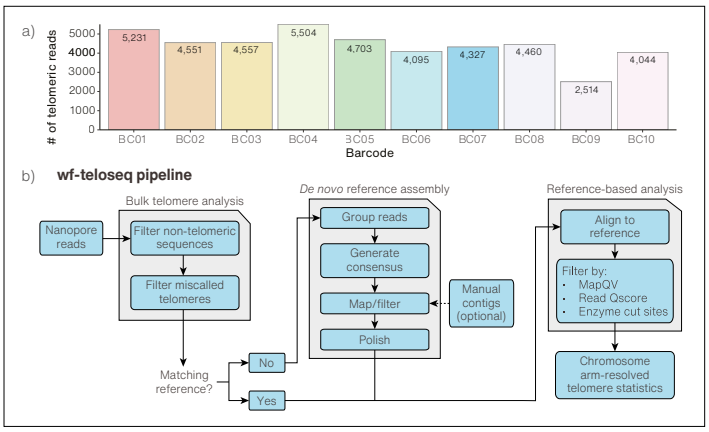

Fig. 2 Telo-seq multiplexing performance and bioinformatics workflow

Nanopore adapters are ligated to the ends of telomeres via splint ligation

Telomeres consist of repetitive motifs ($TTAGGG_n$) with a single-stranded 3' G-rich overhang (Fig. 1a). Telomere length is associated with cellular aging and diseases such as cancer. Many cancer cells activate telomerase, or alternative mechanisms, to maintain telomeres and increase cell survival and propagation. Our approach utilizes the 3' telomeric overhang to ligate an intermediate telomere adapter onto the C-rich strand at the chromosome end (Fig. 1b). The 5' end of the telomere adapter is complementary to the sequencing adapter and allows the C-rich strand to be sequenced in the 5' to 3' direction, from the end of the telomere into the sub-telomeric region.

Telo-seq barcoding enables multiplexed telomere length and chromosome mapping

We multiplexed 10 HG002 Telo-seq samples using MinION™ Flow Cells R10.4.1 for an average of 4,399 mapped telomeric reads per sample (Fig. 2a) with 91/92 chromosome arms assigned. The paternal alleles of chr 22p and 13p are highly homologous and could not be unambiguously mapped. Utilising raw fastqs basecalled with *dorado*, wf-teloseq processes telomeric reads depending on the coverage depth and reference availability (Fig. 2b). The pipeline can measure bulk telomere length or provide higher resolution with a reference-based analysis to resolve chromosome arm mapping and telomere statistics. If a matching reference is unavailable, the pipeline will create a *de novo* reference assembly to continue with a reference-based analysis.

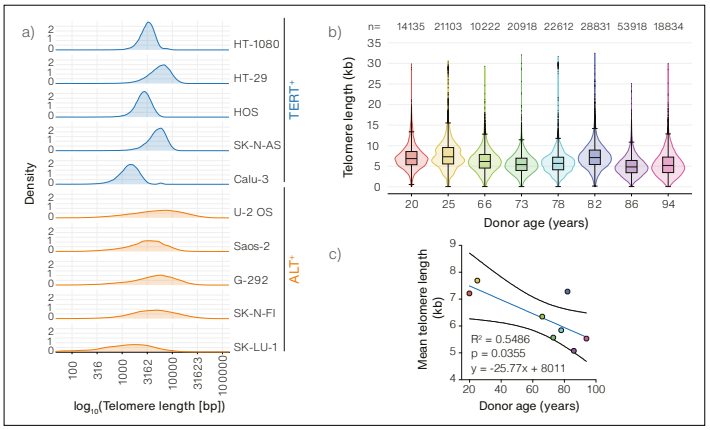

Fig. 3 Telomere lengths in a) cancer cell lines and b,c) donor-derived fibroblasts

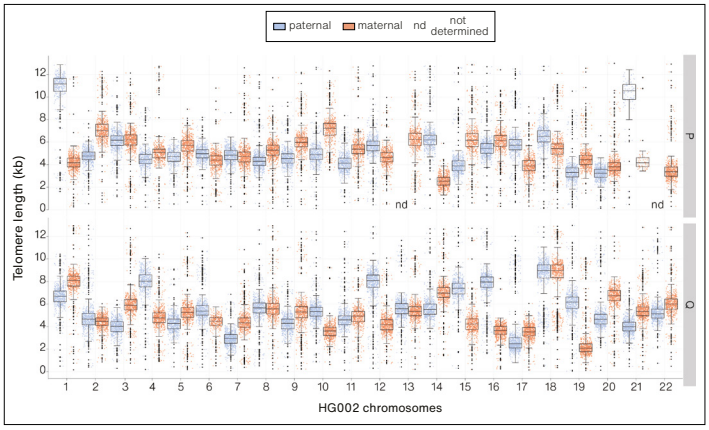

Fig. 4 HG002 telomere lengths by chromosome and haplotype

Telo-seq resolves cancer maintenance pathways and age-related shortening

Cancer cells maintain telomere length by either activating telomerase (TERT+) or alternative lengthening of telomeres (ALT+). Telo-seq can distinguish between five TERT+ and five ALT+ cancer cell lines based on a more tightly distributed average telomere length for TERT+ compared with ALT+ (Fig. 3a). Telo-seq on donor-derived fibroblasts between 20 to 94 years of age revealed shorter telomeres with increased human age, except for an 82-year-old individual who harbored telomeres of comparable length to the young individuals (Fig. 3b). Plotting of the mean telomere length against the donor age and linear regression analysis revealed a general trend of average telomere shortening as a function of donor age (Fig. 3c).

Haplotype-resolved telomere mapping reveals heterogeneity among chromosomes

HG002 telomeric reads were uniquely mapped to a telomere-to-telomere HG002 reference to assess telomeric lengths (Fig. 4). Telomere length heterogeneity is partly a consequence of interchromosome telomere length differences, as well as intrachromosome arm-specific telomere length heterogeneity from the two alleles of each chromosome arm. For example, the HG002 chromosome arm 1p maternal allele had a median telomere length of 4,165 bp, whereas the median paternal allele was 11,139 bp long. The paternal alleles of chromosome 13p and 22p are missing due to not passing the mapping quality filter; however, 86/88 autosomes were correctly mapped.